PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 13 June 2000 (13.06.00)	
International application No. PCT/GB99/03699	**Applicant's or agent's file reference** GWS/20994
International filing date (day/month/year) 05 November 1999 (05.11.99)	**Priority date** (day/month/year) 05 November 1998 (05.11.98)
Applicant SHONE, Clifford, Charles et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

03 May 2000 (03.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03699

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/53 C12N15/62 C12N9/02 A61K38/44 A61K48/00
C07K14/33 A61K39/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FIGUEIREDO D M ET AL: "Delivery of recombinant tetanus -superoxide dismutase proteins to central nervous system neurons by retrograde axonal transport" EXPERIMENTAL NEUROLOGY,US,SAN DIEGO, CA, vol. 145, 1997, pages 546-554, XP002102526 the whole document	1,6,7,9, 10,13,15
Y	FUJII J ET AL: "A defect in the mitochondrial import of mutant Mn-superoxide dismutase produced in Sf21 cells." JOURNAL OF BIOCHEMISTRY, (1998 AUG) 124 (2) 340-6. , XP000867725 page 340 -page 341; figures 1,5	16-22

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 February 2000	15/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Espen, J

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOWLER C ET AL: "Characterization of the Bacillus stearothermophilus manganese superoxide dismutase gene and its ability to complement copper/zinc superoxide dismutase deficiency in Saccharomyces cerevisiae" JOURNAL OF BACTERIOLOGY, vol. 172, no. 3, 1990, pages 1539-1546, XP000877200 USA abstract	16-22
X	FRANCIS JW ET AL: "CuZn superoxide dismutase (SOD-1): tetanus toxin fragment C hybrid protein for targeted delivery of SOD-1 to neuronal cells" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 25, 1995, pages 15434-15442, XP002131795 MD US abstract; figures 1,4-8	1,6,7,9, 10,13,15
X	US 5 780 024 A (BROWN ROBERT H ET AL) 14 July 1998 (1998-07-14) the whole document	1,6,7,9, 10,13,15

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PCT/GB 99/03699

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5780024 A	14-07-1998	NONE	

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
GWS/20994		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/03699	05/11/1999	05/11/1998
International Patent Classification (IPC) or national classification and IPC C12N15/53		
Applicant MICROBIOLOGICAL RESEARCH AUTHORITY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/05/2000	19.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Espen, J Telephone No. +31 70 340 2625

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03699

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-30 as originally filed

Claims, No.:

1-24 as received on 12/12/2000 with letter of 08/12/2000

Drawings, sheets:

1-5 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 11,12.

because:

☒ the said international application, or the said claims Nos. 11,12, with respect to industrial applicability, relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-24
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-24
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-10,13-24
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 11,12 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1). The present application relates to compositions and methods for delivery of superoxide dismutase (SOD), in particular bacterial SOD, to neuronal cells, and in particular to the mitochondria of these cells.

2). Reference is made to the following document:

D1: FIGUEIREDO D M ET AL: 'Delivery of recombinant tetanus - superoxide dismutase proteins to central nervous system neurons by retrograde axonal transport' EXPERIMENTAL NEUROLOGY,US,SAN DIEGO, CA, vol. 145, 1997, pages 546-554, XP002102526

D2: FUJII J ET AL: 'A defect in the mitochondrial import of mutant Mn- superoxide dismutase produced in Sf21 cells.' JOURNAL OF BIOCHEMISTRY, (1998 AUG) 124 (2) 340-6. , XP000867725

3). D1 is regarded as being the closest prior art and discloses a composition for delivery of SOD to neuronal cells, comprising:
- CuZn SOD (D1, p. 551); linked by Gly-Pro-Gly linker to a neuronal cell targeting

component, said component being the C-terminus of the heavy chain (tetanus fragment C (TC)), comprising the determinants for uptake and transport into neuronal cells (D1, p. 546-547, Fig. 1). Said composition was used to be intramuscularly injected in mice.

3.1). Having regard to the available prior art, the claimed matter is novel (Art. 33 (2) PCT).

3.2.1). The subject-matter of claim 1 differs from this known D1 in that the linker is cleavable.

The problem to be solved by the present invention may therefore be regarded as to provide the art with a composition wherein the SOD is released after translocation from the neuronal targeting component.

The solution given in claim 1, i.e. a linker which is cleaved to release SOD, was neither described nor suggested in the closest prior art document.
In consequence, claims 1-17 meet the requirements of Art. 33 (3) PCT.

3.2.2). With respect to claim 18, D2 is regarded of being the closest prior art document, and discloses a polypeptide comprising a human SOD and a sequence for targeting the polypeptide to a human mitochondria.

Claim 18 differs from D2 in that it refers to a polypeptide comprising a bacterial SOD and a sequence for targeting the polypeptide to a human mitochondria.

Such a polypeptide was neither described nor suggested in the closest prior art, and could, therefore, not be deduced in an obvious manner.

In consequence, claims 18-24 meet the requirements of Art. 33 (3) PCT.

3.2.3) The industrial applicability of claims 1-10, and 13-24 is acknowledged (Art. 33 (4) PCT).

4). For the assessment of the present claims 11 and 12 on the question

whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

1). It is clear from the description on page 5 that the following feature is essential to the definition of the invention:

(1) linker is cleavable

Since independent claim 18 does not contain this feature it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

2). Although claims 1 and 18 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought ..and/or.. in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 1 and 18 do not meet the requirements of Article 6 PCT.

3). The cleavable linker of claims 1 and 16 should be characterized by true technical features (e.g. claims 2 and 17) (Art. 6 PCT).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
GWS/20994		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/03699	05/11/1999	05/11/1998
International Patent Classification (IPC) or national classification and IPC C12N15/53		
Applicant MICROBIOLOGICAL RESEARCH AUTHORITY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/05/2000	19.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Espen, J Telephone No. +31 70 340 2625

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/03699

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-30	as originally filed				

Claims, No.:

1-24	as received on	12/12/2000	with letter of	08/12/2000	

Drawings, sheets:

1-5	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

- ☐ the entire international application.
- ☒ claims Nos. 11,12.

because:

☒ the said international application, or the said claims Nos. 11,12, with respect to industrial applicability, relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-24
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-24
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-10,13-24
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 11,12 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1). The present application relates to compositions and methods for delivery of superoxide dismutase (SOD), in particular bacterial SOD, to neuronal cells, and in particular to the mitochondria of these cells.

2). Reference is made to the following document:

D1: FIGUEIREDO D M ET AL: 'Delivery of recombinant tetanus - superoxide dismutase proteins to central nervous system neurons by retrograde axonal transport' EXPERIMENTAL NEUROLOGY,US,SAN DIEGO, CA, vol. 145, 1997, pages 546-554, XP002102526

D2: FUJII J ET AL: 'A defect in the mitochondrial import of mutant Mn- superoxide dismutase produced in Sf21 cells.' JOURNAL OF BIOCHEMISTRY, (1998 AUG) 124 (2) 340-6. , XP000867725

3). D1 is regarded as being the closest prior art and discloses a composition for delivery of SOD to neuronal cells, comprising:
- CuZn SOD (D1, p. 551); linked by Gly-Pro-Gly linker to a neuronal cell targeting

component, said component being the C-terminus of the heavy chain (tetanus fragment C (TC)), comprising the determinants for uptake and transport into neuronal cells (D1, p. 546-547, Fig. 1). Said composition was used to be intramuscularly injected in mice.

3.1). Having regard to the available prior art, the claimed matter is novel (Art. 33 (2) PCT).

3.2.1). The subject-matter of claim 1 differs from this known D1 in that the linker is cleavable.

The problem to be solved by the present invention may therefore be regarded as to provide the art with a composition wherein the SOD is released after translocation from the neuronal targeting component.

The solution given in claim 1, i.e. a linker which is cleaved to release SOD, was neither described nor suggested in the closest prior art document.
In consequence, claims 1-17 meet the requirements of Art. 33 (3) PCT.

3.2.2). With respect to claim 18, D2 is regarded of being the closest prior art document, and discloses a polypeptide comprising a human SOD and a sequence for targeting the polypeptide to a human mitochondria.

Claim 18 differs from D2 in that it refers to a polypeptide comprising a bacterial SOD and a sequence for targeting the polypeptide to a human mitochondria.

Such a polypeptide was neither described nor suggested in the closest prior art, and could, therefore, not be deduced in an obvious manner.

In consequence, claims 18-24 meet the requirements of Art. 33 (3) PCT.

3.2.3) The industrial applicability of claims 1-10, and 13-24 is acknowledged (Art. 33 (4) PCT).

4). For the assessment of the present claims 11 and 12 on the question

whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII
Certain observations on the international application

1). It is clear from the description on page 5 that the following feature is essential to the definition of the invention:

(1) linker is cleavable

Since independent claim 18 does not contain this feature it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

2). Although claims 1 and 18 have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought ..and/or.. in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of the protection. Hence, claims 1 and 18 do not meet the requirements of Article 6 PCT.

3). The cleavable linker of claims 1 and 16 should be characterized by true technical features (e.g. claims 2 and 17) (Art. 6 PCT).

CLAIMS

1. A composition for delivery of superoxide dismutase (SOD) to neuronal cells, comprising:-

SOD; linked by a cleavable linker to

a neuronal cell targeting component, comprising a first domain that binds to a neuronal cell and a second domain that translocates the SOD of the composition into the neuronal cell, wherein, after translocation of the SOD into the cell, the linker is cleaved to release SOD from the neuronal cell targeting component.

2. A composition according to Claim 1 wherein the cleavable linker is:-

- a disulphide bridge between cysteine residues, one residue on the SOD and one residue on the neuronal cell targeting component; or
- a site for a protease found in neuronal cells.

3. A composition according to Claim 1 or 2 for delivery of SOD to mitochondria of neuronal cells wherein the SOD comprises a sequence targeting the SOD to mitochondria in the neuronal cell.

4. A composition according to Claim 3 wherein the SOD is a hybrid of Mn-SOD and a sequence targeting the hybrid to mitochondria.

5. A composition according to Claim 3 or 4 wherein the mitochondria targeting sequence is derived from human Mn-SOD.

6. A composition according to any of Claims 1-5 wherein the SOD is bacterial SOD or is a derivative thereof that substantially retains the superoxide dismutase activity of bacterial SOD.

7. A composition according to any of Claims 1 to 6 wherein the first domain is selected from (a) neuronal cell binding domains of clostridial toxins; and (b) fragments, variants and derivatives of the domains in (a) that substantially retain the neuronal cell binding activity of the domains of (a).

8. A composition according to any Claims 1 to 7 wherein the second domain is selected from (a) domains of clostridial neurotoxins that translocate polypeptide sequences into cells, and (b) fragments, variants and derivatives of the domains of (a) that substantially retain the translocating activity of the domains of (a).

9. A composition according to any of Claims 1 to 8 wherein the linker is a disulphide bridge.

10. A pharmaceutical composition for treatment of oxidative damage to neuronal cells comprising a composition according to any of Claims 1 to 9 and a pharmaceutically acceptable carrier.

11. A method of delivering SOD to a neuronal cell comprising administering a composition according to Claim 10.

12. A method according to Claim 11 comprising injecting the composition.

13. A method of making a composition according to any of Claims 1 to 8 comprising chemically linking SOD, a linker and a neuronal cell targeting component.

14. A method of making a composition according to any of Claims 1 to 9 comprising expressing a DNA that codes for a polypeptide having SOD activity, a linker, and a neuronal cell targeting component.

15. A method according to claim 14 wherein the polypeptide further comprises a purification sequence and the method further comprises purifying the polypeptide and then cleaving the polypeptide to remove the purification sequence to leave SOD, the linker and the neuronal cell targeting component.

16. A composition for delivery of a therapeutic agent to neuronal cells, comprising:-

the therapeutic agent; linked by a cleavable linker to
a neuronal cell targeting component, comprising a first domain that binds to a neuronal cell and a second domain that translocates the therapeutic agent of the composition into the neuronal cell wherein, after translocation of the SOD into the cell, the linker is cleaved to release SOD from the neuronal cell targeting component.

17. A composition for delivery of a therapeutic agent to neuronal cells according to Claim 16, wherein the cleavable linker is either a disulphide bridge or a site for a protease found in neuronal cells.

18. A polypeptide comprising a bacterial SOD or derivative thereof that substantially retains the superoxide dismutase activity of bacterial SOD and a sequence for targeting the polypeptide to a human mitochondria.

19. A polypeptide according to Claim 18 wherein the SOD is from *Bacillus*.

20. A polypeptide according to Claim 18 or 19 which is a fusion protein.

21. A nucleotide encoding the polypeptide of any of Claims 18-20.

22. A vector comprising the nucleotide of Claim 21.

23. A method of making a polypeptide according to any of Claims 18-20 comprising expressing the nucleotide sequence of Claim 21.

24. A cell comprising the nucleotide sequence of Claim 21 or the vector of Claim 22.

ATENT COOPERATION TREAT

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
GWS/20994		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 03699	05/11/1999	05/11/1998
Applicant		
MICROBIOLOGICAL RESEARCH AUTHORITY et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No. 3

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/03699

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/53 C12N15/62 C12N9/02 A61K38/44 A61K48/00
C07K14/33 A61K39/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FIGUEIREDO D M ET AL: "Delivery of recombinant tetanus -superoxide dismutase proteins to central nervous system neurons by retrograde axonal transport" EXPERIMENTAL NEUROLOGY,US,SAN DIEGO, CA, vol. 145, 1997, pages 546-554, XP002102526 the whole document	1,6,7,9, 10,13,15
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[X] Further documents are listed in the continuation of box C.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOWLER C ET AL: "Characterization of the Bacillus stearothermophilus manganese superoxide dismutase gene and its ability to complement copper/zinc superoxide dismutase deficiency in Saccharomyces cerevisiae" JOURNAL OF BACTERIOLOGY, vol. 172, no. 3, 1990, pages 1539-1546, XP000877200 USA abstract	16-22
X	FRANCIS JW ET AL: "CuZn superoxide dismutase (SOD-1): tetanus toxin fragment C hybrid protein for targeted delivery of SOD-1 to neuronal cells" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 25, 1995, pages 15434-15442, XP002131795 MD US abstract; figures 1,4-8	1,6,7,9, 10,13,15
X	US 5 780 024 A (BROWN ROBERT H ET AL) 14 July 1998 (1998-07-14) the whole document	1,6,7,9, 10,13,15

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/03699

Patent document cited in search report	Publication dat	Patent family member(s)	Publication date
US 5780024 A	14-07-1998	NONE	

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(71) Applicant *(for all designated States except US)*: MICROBIOLOGICAL RESEARCH AUTHORITY [GB/GB]; CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG (GB).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SHONE, Clifford, Charles [GB/GB]; Microbiological Research Authority, CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG (GB). SUTTON, John, Mark [GB/GB]; Microbiological Research Authority, CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG (GB). HALLIS, Bassam [GB/GB]; Microbiological Research Authority, CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG (GB). SILMAN, Nigel [GB/GB]; Microbiological Research Authority, CAMR, Porton Down, Salisbury, Wiltshire SP4 0JG (GB).

(74) Agents: SCHLICH, George, William et al.; Mathys & Squire, 100 Gray's Inn Road, London WC1X 8AL (GB).

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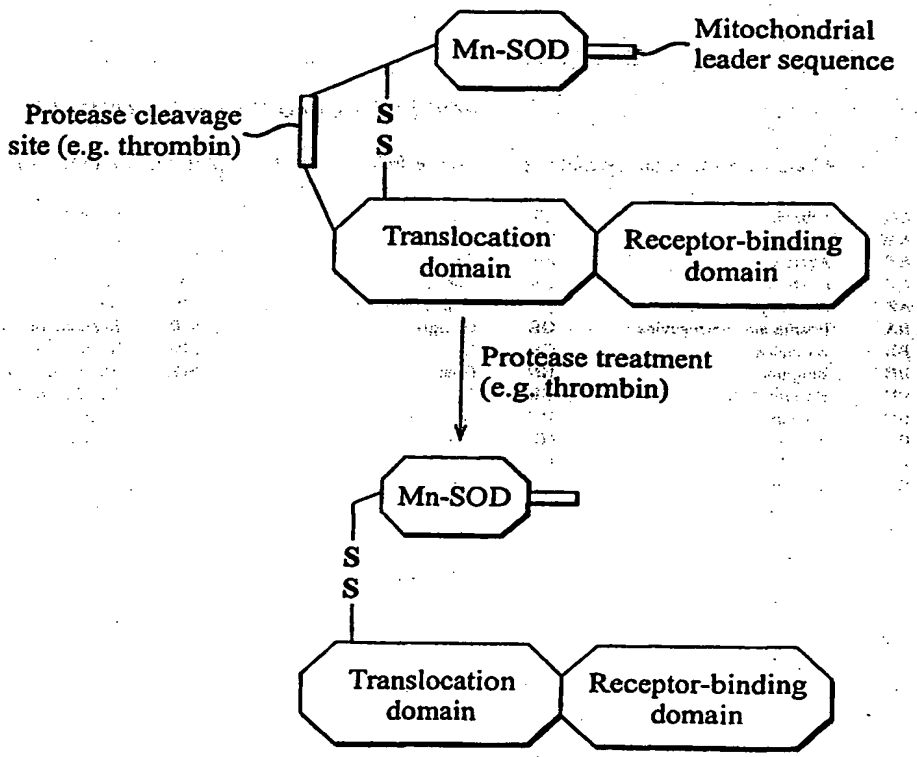
(54) Title: DELIVERY OF SUPEROXIDE DISMUTASE TO NEURONAL CELLS

(57) Abstract

A composition for delivery of superoxide dismutase to neuronal cells comprise a superoxide dismutase linked by a linker to a neuronal cell targeting component, which component comprises a first domain that binds to a neuronal cell and a second domain that translocates the superoxide dismutase into the neuronal cell. After translocation, the linker is cleaved to release superoxide dismutase from the neuronal cell targeting domain. Also described is use of the composition for treatment of oxidative damage to neuronal cells and further targeting of the composition using human mitochondrial leader sequences. A hybrid polypeptide is described that contains a bacterial superoxide dismutase plus a sequence that targets a human mitochondria.



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CLAIMS

1. A composition for delivery of superoxide dismutase (SOD) to neuronal cells, comprising:-

SOD; linked by a cleavable linker to

a neuronal cell targeting component, comprising a first domain that binds to a neuronal cell and a second domain that translocates the SOD of the composition into the neuronal cell.

2. A composition according to Claim 1 for delivery of SOD to mitochondria of neuronal cells wherein the SOD comprises a sequence targeting the SOD to mitochondria in the neuronal cell.

3. A composition according to Claim 2 wherein the SOD is a hybrid of Mn-SOD and a sequence targeting the hybrid to mitochondria.

4. A composition according to Claim 2 or 3 wherein the mitochondria targeting sequence is derived from human Mn-SOD.

5. A composition according to any of Claims 1-4 wherein the SOD is bacterial SOD or is derived therefrom.

6. A composition according to any of Claims 1 to 5 wherein the first domain is selected from (a) neuronal cell binding domains of clostridial toxins; and (b) fragments, variants and derivatives of the domains in (a) that substantially retain the neuronal cell binding activity of the domains of (a).

7. A composition according to any Claims 1 to 6 wherein the second domain is selected from (a) domains of clostridial neurotoxins that translocate polypeptide sequences into cells, and (b) fragments, variants and derivatives of the domains of (a) that substantially retain the

translocating activity of the domains of (a).

8. A composition according to any of Claims 1 to 7 wherein the linker is a disulphide bridge.

9. A pharmaceutical composition for treatment of oxidative damage to neuronal cells comprising a composition according to any of Claims 1 to 8 and a pharmaceutically acceptable carrier.

10. A method of delivering SOD to a neuronal cell comprising administering a composition according to Claim 9.

11. A method according to Claim 10 comprising injecting the composition.

12. A method of making a composition according to any of Claims 1 to 8 comprising chemically linking SOD, a linker and a neuronal cell targeting component.

13. A method of making a composition according to any of Claims 1 to 8 comprising expressing a DNA that codes for a polypeptide having SOD activity, a linker, and a neuronal cell targeting component.

14. A method according to claim 13 wherein the polypeptide further comprises a purification sequence and the method further comprises purifying the polypeptide and then cleaving the polypeptide to remove the purification sequence to leave SOD, the linker and the neuronal cell targeting component.

15. A composition for delivery of a therapeutic agent to neuronal cells, comprising:-

the therapeutic agent; linked by a cleavable linker to a neuronal cell targeting component, comprising a first domain that binds to a neuronal cell and a second domain that translocates the therapeutic agent of the composition into the neuronal cell.

16. A polypeptide comprising a bacterial SOD or derivative thereof and a sequence for targeting the polypeptide to a human mitochondria.

17. A polypeptide according to Claim 16 wherein the SOD is from *Bacillus*.

18. A polypeptide according to Claim 16 or 17 which is a fusion protein.

19. A nucleotide encoding the polypeptide of any of Claims 16-18.

20. A vector comprising the nucleotide of Claim 19.

21. A method of making a polypeptide according to any of Claims 16-18 comprising expressing the nucleotide sequence of Claim 19.

22. A cell comprising the nucleotide sequence of Claim 19 or the vector of Claim 20.